LOTHIAN UNIVERSITY HOSPITALS DIVISION

DEPARTMENT OF LABORATORY MEDICINE

Scottish Mycobacteria Reference Laboratory & Lothian Mycobacteria Laboratory

User Manual

August 2013

CPA Accredited

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Scottish Mycobacteria Reference Laboratory

The Scottish Mycobacteria Reference Laboratory (SMRL) is the Scottish Reference Laboratory for Mycobacteriology, particularly for the identification, drug susceptibility testing and strain typing of *Mycobacterium tuberculosis* complex (MTBC) and non-tuberculous mycobacteria (NTM). The laboratory receives approximately 1,000 mycobacterial cultures per year mainly from Scottish NHS laboratories. The service is also funded to perform rapid detection of mycobacteria and screening for drug resistance on acid-fast bacilli (AFB) smear positive respiratory samples. Further advice is also available as detailed on page 13.

The laboratory is based within the Royal Infirmary of Edinburgh, Little France Crescent, Edinburgh, EH16 4SA. It is fully integrated with the combined laboratories of the University Hospitals Division of NHS Lothian. SMRL is funded through an agreement between National Service Scotland and NHS Lothian University Hospitals Division. Some services have been developed which are available at a charge to the user.

This manual covers three levels of service:

1. National Reference services funded by National Services Scotland (NSS).
2. Services paid for by charging the users per test.
3. 'Local' services funded by NHS boards currently NHS Lothian, NHS Borders, NHS Dumfries and Galloway, NHS Highland and NHS Tayside.

Telephone, Fax and Email Enquiries

TB Laboratory Tel: 0131 242 6022/23
TB Office Tel: 0131 242 6016
Confidential Fax in Microbiology Office: 0131 242 6008
Shona.Hannan@luht.scot.nhs.uk
Michael.Smith@luht.scot.nhs.uk

Core hours

The core laboratory hours are 9am to 5pm, Monday to Friday, but not weekends, Christmas and New Year holidays. At other times, Lothian laboratory users can discuss urgent AFB microscopy via the Consultant/Specialist Registrar on-call for clinical bacteriology (contact RIE switchboard Tel: 0131 536 1000). There is no out-of-hours AFB screening or Reference Laboratory service. For urgent enquiries out-of-hours, the on call Consultant Microbiologist may be contacted through the RIE switchboard (Tel 0131 536 1000) in the first instance.

Contact list

Name and Designation	Telephone Numbers and e-mail Address
Mrs Shona Hannan Reference Laboratory Administrator	Tel 0131 242 6016 Shona.Hannan@luht.scot.nhs.uk
Dr Ian Laurenson Director	Tel: 0131 242 6079 Ian.Laurenson@luht.scot.nhs.uk
Dr Ewan Olson Deputy Director	Tel: 0131 242 6072 Ewan.Olson@luht.scot.nhs.uk
Mr Mike Smith BMS Team Manager	Tel: 0131 242 6009 Michael.Smith@luht.scot.nhs.uk
Mrs Pauline Claxton BMS Section Manager	Tel: 0131 242 6009 Pauline.Claxton@luht.scot.nhs.uk
Mrs Christine Doig BMS Section Manager	Tel: 0131 242 6009 Christine.Doig@luht.scot.nhs.uk
BMS Specialists Mr Gary Davidson Mr Lukman Elabor Mrs Fiona Mathewson Ms Sandra Houston	Tel: 0131 242 6022/6023
Mr Neil McMinn MTO	
Mrs Jane Greig Advanced BSW	
Dr Louise Seagar Clinical Scientist	Tel: 0131 242 6009 Louise.Seagar@luht.scot.nhs.uk

The service is managed overall by NHS Lothian. If we are unable to deal with an issue and you wish to discuss a matter with NHS Lothian Management then contact:

Mr Ian King
Tel: 0131 242 7051
mailto:Ian.King@luht.scot.nhs.uk
Healthcare Science Manager, Cell Sciences, NHS Lothian.

Packing and Transport of Infectious Substances

Postal Address

Scottish Mycobacteria Reference Laboratory
Department of Laboratory Medicine
Royal Infirmary of Edinburgh
51 Little France Crescent
Old Dalkeith Road
Edinburgh EH16 4SA

DX Address

Exchange: Edinburgh 96 EH
DX Number: DX6231201

NB Infectious substances may only be transported in packaging, which meets the U.N. Class 6.2 specifications and the 602 packing requirements. All Cultures must be sent by DX NOT by post.

Packaging Mycobacteria Cultures or Samples:

1. Remove any water of condensation from the solid culture vial(s) - taking normal safety precautions for handling cultures.
2. Seal the cap with 'parafilm'.
3. Wrap individual vials in sufficient absorbent material to absorb all the liquid in the vial and to protect it from breakage.
4. Seal this package twice in plastic bags.

For posting by First Class Post (Samples Only):

1. Place the wrapped sample inside the inner container of the packaging and tighten the lid.
2. Place the inner container into the outer cardboard box..
3. Close the box and secure with tape.
4. Stick address label on the top of the box.
5. Stick "From" label, with senders address, on the bottom of the box
6. Post by First Class Post or courier such as DX for samples. Cultures must be sent by DX or equivalent to conform to the Transport of Dangerous Goods act. The SMRL is a member of the Hays DX Specimen Delivery Service.

For posting by Hays DX courier:

1. Place the sealed specimen bag into a screw-cap container from a DX box and put with it the corresponding request forms.
2. Carefully place the destination address label provided on the green tracking label on the package taking care not to cover any of the peel-off barcodes or tracking numbers. Also leave some space next to the lines for receiver's Hays DX number and Exchange code. Place some clear selotape over the label to ensure it is not easily removed or damaged by rain, again avoiding covering any peel-off labels.
3. Fill in the Hays DX number and Exchange code of the destination.
4. Peel off **one** of the two matching tracking numbers at the bottom of the Tracked Specimen label and stick it in the provided Hays DX logbook and fill in the receiver's details and date of dispatch.
5. At the top of the Hays DX label, fill in the sender's name, sender's Hays DX number, sender's exchange code and the name and contact number for the sender. This information is written on a separate card at the front of the Hays DX logbook,
6. Place the provided blue security seal sticker over the package closure and dispatch.

Levels of Service

1. National Services Scotland funded Remit

SMRL receives cultures of presumed mycobacteria from all NHS laboratories in Scotland. These cultures are tested to determine

- Presence of mycobacteria.
- Full species identification.
- Susceptibility to standard anti-mycobacterial drugs.
- Susceptibility to second line anti-mycobacterial drugs where appropriate.
- Rapid liquid culture of smear positive respiratory samples.
- Molecular typing and comparison of *M. tuberculosis* complex strains.
- Use of molecular tests on smear positive respiratory samples to rapidly identify the presence of mycobacteria and screen for drug resistance in *M.tuberculosis* complex.

2. Services available by charging users.

- Rapid liquid culture of samples from normally sterile sites (e.g. CSF, bone marrow, tissue samples, aspirates and bronchoalveolar lavage samples).
- Molecular tests on smear negative samples and smear positive non-respiratory samples.
- Culture of smear negative samples.
- PCR for molecular detection of MTB complex in fixed paraffin sections.

3. Services available by contract between requesting service and NHS Lothian

- Processing of routine samples from patients for mycobacteria investigation.

Samples for mycobacterial investigations

<u>Microscopy</u>
All samples except blood and urine are routinely examined for direct AFB microscopy (provided there is sufficient specimen).

<u>Culture</u>
Our current practice is to inoculate all specimens into liquid and solid media.

<u>Drug susceptibility testing</u>
Susceptibilities are routinely performed on the first isolate from each patient and then only repeated at 3 month intervals.

<u>Molecular tests</u>
Smear positive respiratory samples are tested by rapid molecular tests if sufficient quantity is received.

<u>Immunology:Interferon Gamma Release Assay (IGRA)</u>
Testing is not currently performed' in-house. Alternative providers for IGRA testing using the Quantiferon-TB Gold In-Tube Assay are:

1. National Mycobacterium Reference Laboratory
Abernethy Building
Institute of Cell and Molecular Science (ICMS)
2 Newark Street,
Whitechapel
London
E1 2AT
Tel: 020 7377 5895
mailto:v.nikolayevskyy@qmul.ac.uk

2. Newcastle HPA Regional Mycobacterial Reference Laboratory
Level 2
Freeman Hospital
High Heaton
Newcastle upon Tyne
NE7 7DN
Tel: 0191 282 1150
anne.barrett@hpa.org.uk

The T-spot assay is another IGRA, which is available from:

Oxford Immunotec
115D Milton Park
Abingdon
Oxfordshire
OX14 4RZ
Tel: +44 (0) 1235 442 780
Fax: +44 (0) 1235 442 781
www.oxfordimmunotec.com

Users should inform us in advance of cultures or specimens that are being sent by post or courier. A staff member of the SMRL should be contacted by phone (see contact list above) or by fax (0131 242 6008). We will issue a receipt or report within one working day from receipt of cultures or samples.

A completed SMRL request form (see page 16) **must** accompany every culture or specimen. Wherever possible, please time the posting of samples and cultures to minimise the possibility of these lying unattended over weekends and holidays.

All requests must provide the following Minimum Data Set information:

Forms

1. Patient Identifier Number (CHI-for Scottish patients)
2. Postcode of patient where available
3. Sender's Laboratory Number
4. Surname
5. Forename
6. Date of Birth
7. Gender
8. Location of Sender
9. Specimen type (or, if culture isolate, specimen type from which the culture was isolated).

10. Any relevant clinical information. If results need to be telephoned please supply contact details.
11. If possible patient's hospital and consultant.

Sample

Minimum data are also required on the sample container:

1. Surname
2. Forename
3. Date of Birth
4. Sample date and time
5. Sample type and site
6. Location of patient
7. Sending Laboratory number if applicable

Vials

On culture vials, patient name and the sender's Laboratory Number are required as a minimum.

Sample types and containers for mycobacteria investigation

Specimen type	Containers and transport	Comments
Sputum (up to 5ml)	Disposable plastic, sterile "universal" container	Decontaminated and neutralised samples may lose viability during transit
Induced sputum	Disposable plastic, sterile "universal" container	
Bronchoalveolar lavage (BAL)/ Bronchial Washings	Disposable plastic, sterile "universal" container	
Pleural fluids & aspirates	Disposable plastic, sterile "universal" container	
Biopsy material	Disposable plastic, sterile "universal" container (add a few drops of preservative free 0.9% sterile saline to keep sample moist)	DO NOT USE FORMOL SALINE OR FORMALIN
Tissues (including heart valves)	Disposable plastic, sterile "universal" container	DO NOT USE FORMOL SALINE OR FORMALIN
Early Morning Urine	Sterile 500ml bottles filled up to 2/3 full. Send three samples on successive days.	EMU bottles available on request from the stores department, Astley Ainsley Hospital, Edinburgh for Lothian users.
Blood	Myco F lytic Blood culture bottles available from SMRL	
Bone marrow	Use Myco F lytic blood culture bottles available from SMRL or disposable plastic, sterile "universal" container	
CSF	Disposable plastic, sterile "universal" container	Recommended minimum volume of 6mls
Stools	Disposable plastic, sterile "universal" container with spoon	
PM specimens	Disposable plastic, sterile "universal" container or larger screw capped sterile container if appropriate	DO NOT USE FORMOL SALINE OR FORMALIN
Cultures	Egg medium slopes or aliquots of liquid medium	At least 5mls required if grown in liquid medium
Paraffin sections for PCR	Block/section sliced in uncontaminated manner	

Tests available, turn around times and costs

Turnaround times for cultures are dependent on receipt of a pure culture containing sufficient mycobacteria for analysis.

The time interval between receipt of a culture and the issue of the final identification and first line sensitivity report varies greatly from 1-12 weeks, depending on factors such as the nature of the culture medium used by the sending laboratory, paucity of organisms in the culture, the species of Mycobacteria and the presence or absence of contamination.

The costs for NHS users indicated on page 11 will be reviewed annually. They may vary without notice. Costs for private tests are available on request.

Test	Method	Test frequency and turn around time	Comments	Costs
Microscopy for acid-fast bacilli (AFB) detection	Auramine Phenol staining	Within 1 working day of receipt (Mon-Fri)		All specimens examined by AP microscopy are cultured for mycobacteria. Costs indicated below against "Primary culture" include primary specimen microscopy
Primary Culture	Solid (modified LJ pyruvate egg slope) and rapid liquid culture (MGIT)	Daily. Usually available 2-6 weeks after receipt of sample. Cultures are reported negative at 8 weeks if appropriate but kept for 12 weeks in total.	Results from contaminated specimens may be delayed	£25.50 for NHS users (Free for NHS Lothian users)
Species identification of cultures	Molecular identification methods	Twice weekly (Tues/Fri); 1-7 days	Additional molecular or phenotypic methods may be required for some strains, and this may cause delay	
Drug susceptibility testing of slow growing mycobacteria	Liquid or solid culture (broth microdilution for MOTT)	Set up daily or weekly depending on methodology	Tests for 2nd and 3rd line TB drugs set up on request or when there is resistance to any first line drug	
Drug susceptibility testing of "Rapid Growers"	Broth microdilution	As required; 3- days incubation	For rapidly growing mycobacteria	
Rapid molecular detection of MTBC	Real Time PCR	Weekly (Tuesday pm/Wednesday pm)	Test is only validated for respiratory samples	First smear +ve respiratory samples are free to NHS Scotland users otherwise the charge is £130
Rapid rifampicin and isoniazid resistance detection	Genotype MTBDR plus	Weekly (Wednesday pm/Thursday am)		
Molecular genotyping of *M. tuberculosis* Currently 24 locus MIRU-VNTR. Newer technologies such as next generation sequencing may become available shortly		Weekly	All new TB patients' isolates are typed.	
Media QC		On request; 6-8 weeks		
Paraffin sections for molecular detection of MTBC	Real Time PCR	Weekly (Tuesday pm/Wednesday pm) on request		£78 for NHS users

Molecular detection of mycobacteria in specimens

Note on rapid molecular tests: False negatives and false positives can and do occur.

We currently use an in-house real-time PCR for the direct detection of MTBC in specimens and we continue to use the Genotype MTBDR*plus* kit (Hain Lifescience) for the direct detection of rifampicin and isoniazid resistance in samples. From time to time alternative methods such as the Cepheid X-Pert may be used.

Direct detection of MTBC from formalin fixed tissues specimens is available by arrangement. Results have to be interpreted with caution. Real-time PCR using FFPE tissue is a developmental test for which limited technical validation has been performed. Accuracy is partly dependant on avoiding laboratory cross contamination not only in microbiology and the PCR suite but also in histopathology. Results to date suggest approximately 66% of MTB complex will be detected by this method as compared with concurrent mycobacterial culture. FFPE specimens are not suitable for culture.

Reporting of Results

Policy and procedures for telephoned results
Previously unknown AFB smear positive or discrepant samples are telephoned as soon as possible, usually within 1 working day of receipt. New positive cultures obtained from smear negative samples are also phoned as soon as possible after the presence of mycobacteria is confirmed in the culture.

- The following examples will also be telephoned:
- results of direct rapid molecular tests on specimens if findings are unexpected.
- clinically important identification or drug susceptibility test results (e.g. primary resistance to first line drugs; acquired new resistance; unexpected mycobacterial species).
- new positive cultures from smear negative specimen/blood.

Emailing of reports to secure nhs.net accounts is set up already for some user laboratories. In some instances no other written report is sent out. We can set this up if you have such a secure laboratory address to automatically email each time there is an updated report. Please contact Mike Smith or Shona Hannan in the first instance if you wish to use this means of reporting.

Written report formats

Initial Report on specimens Microscopy results Acid Fast Bacilli NOT seen Culture report to follow Microscopy results Few Acid Fast Bacilli seen Culture report to follow
Initial Reports on Cultures received Confirmation as a Mycobacterium, and provisional species identification is reported in writing within 4 working days of receipt of culture. This is achieved by AFB microscopy to confirm the presence of mycobacteria and rapid molecular methods for identification of cultures. **Final Report on Cultures received** Confirmed species identification and first line sensitivity results may take from as little as one week to up to 12 weeks. **Molecular Typing** *M. tuberculosis* genotyping results are reported as they are done and comparisons of the genotypes of strains suspected to be clustered are reported to CPHM's quarterly at present. Further comment/analysis is available on discussion with a senior member of SMRL staff and a letter may be issued. **Paraffin sections** Paraffin embedded tissues can be examined for the molecular detection of MTBC. Results are normally reported within 5 working days. **Cumulative reports** Cumulative results on a patient with positive results can be produced on request. Please contact senior BMS or medical staff at SMRL.
At present only Authorised Final reports on each 'test' are available on SCI store. Interim results are not available.

Technical, Scientific and Health and Safety advice

The technical and scientific staff at the SMRL are happy to provide advice on all laboratory aspects of microscopy and isolation of Mycobacteria and on the safe running and design of containment level 3 laboratories.

Please contact Mr Mike Smith (BMS Team Manager), Mrs Pauline Claxton or Mrs Christine Doig (BMS Section Managers') for technical help and advice.

Clinical, epidemiological and infection control advice

The primary source of medical advice for the diagnosis, management and public health investigation of TB lies with the consultants in the relevant specialties in the local health board. The

consultant microbiologists attached to the SMRL are happy to complement this primary advice where appropriate.
Please contact Dr Ian Laurenson (Director) or Dr Ewan Olson (Deputy Director) for advice on issues such as microbiological diagnostic tests, antibiotic susceptibility tests, strain typing for contact tracing, and infection control.

Training and Education

Audit Study Day
Every two years the laboratory hosts an Audit/Study day to allow SMRL staff to meet and discuss various issues with reference laboratory users, public health consultants, chest physicians and Health Protection Scotland (HPS) representatives. The last meeting was held in June 2012. The next meeting will be held in 2014.

Attachments and visits
Each year SMRL provides training attachments for staff from other laboratories including Biomedical Scientists, Clinical Scientists, and Trainee Doctors. SMRL also provides support for undergraduate science and medical students, MSc students and projects for Trainee Doctors.

Visits and lectures by SMRL staff to other institutions
Staff at SMRL provide teaching and advice at local and national level on invitation particularly in the areas of diagnosis, patient management and molecular epidemiology.

Contact details
To discuss short visits to SMRL for training purposes, please contact Mr. Mike Smith, BMS Team Manager in the first instance.

Research and Audit

Staff are willing to collaborate in relevant audit and research activities. This may take the form of simple data searches to more formal grant applications dependent on ethical approval where relevant and availability of resources.

Contact details
Please contact Dr Ian Laurenson (Director), Dr Ewan Olson (Deputy Director) or Dr Louise Seagar, Clinical Scientist for discussions regarding research/audit collaboration.

Quality Assurance

The SMRL is committed to high quality laboratory analysis. The laboratory participates in the UK National External Quality Assurance (NEQAS) scheme for Mycobacteria microscopy, culture and molecular typing as well as Quality Control for Molecular Diagnostics (QCMD) for molecular detection in specimens. The laboratory also participates in the WHO ‘Drug Proficiency Testing Scheme’, an external QA scheme for sensitivity testing against first line anti-tuberculous drugs. We also carry out Internal Quality Assurance (IQA).

SMRL is fully CPA accredited.

The SMRL Annual Report and User Manual are available on the HPS website.

Antimicrobial Assays.

The following information is for assays on first line TB therapy drugs. Information on other anti mycobacterial drugs can be found at http://www.assayfinder.com/

Isoniazid, Rifampicin and Ethambutol

Provider	Cardiff Toxicology Laboratories
Contact Name	Dr. Alan Hutchings
Address For Specimens	Toxicology Laboratory The Academic Centre Llandough Hospital Penarth CF64 2XX
Country	United Kingdom
Tel. No. (Voice)	029 2071 6893
Tel. No. (Fax)	029 2035 0142
E Mail (click to mail)	HutchingsAD@cf.ac.uk
Accreditation Status	Accredited
Other Information	The Therapeutics & Toxicology Centre is a WDA Centre of Expertise
Web Site (click to view)	http://www.ctlabs.co.uk

Pyrazinamide

No longer provided due to closure of the London, Medical Toxicology Unit at St Thomas Hospital.

Rifampicin

Provider	BCARE, Antimicrobial Reference Laboratory, Southmead Hospital
Contact Name	Dr AM Lovering
Address For Specimens	ARL Department of Microbiology Southmead Hospital Westbury on Trym Bristol BS10 5NB
Country	United Kingdom
Tel. No. (Voice)	0117 959 5653
Tel. No. (Fax)	0117 959 3217
E Mail (click to mail)	andrew.lovering@nbt.nhs.uk
Accreditation Status	Accredited
Other Information	
Web Site (click to view)	http://www.bcare.nbt.nhs.uk/services/clinical-antimicrobial-assays

Flag: -……TBR No……Received Date……SMRL Lab No……

SCOTTISH MYCOBACTERIA REFERENCE LABORATORY – FORM

Microbiology Laboratory
Royal Infirmary of Edinburgh
Little France
51 Little France Crescent
Edinburgh
EH16 4SA

Telephone numbers: -
Enquiries 0131-242-6016
Dr. Ian F Laurenson 0131-242-6079
Mike Smith BMS Team Manager 0131-242-6009
Laboratory 0131-242-6022
Fax: - 0131-242-6008

Patient's Surname ....Forename .. .. Sex M / F
Home Address ....Date of Birth ..... /...../ .....
....Postcode ....
Country of Origin ....Race or Ethnic Group ....
Hospital or Clinic No. ... .Laboratory Reference No ....
CHI Number ....
Patient's Hospital……Patient's Consultant……
Sending Laboratory..... ....
Date of specimen (or received date) ... . /...../ ..... AFB Microscopy of specimen POS / NEG

Sample Type:- Positive Culture ☐ or Primary Specimen ☐
Specimen type/Isolation site..... ....
Antimicrobials at the time of sampling..... ....
Comments From Local Laboratory. ……. ……. ……. ……. ……. ……. ……. ……. ……. ……. .....……
…. …… …….. …….. …….. …….. …….. …….. …….. …….. …….. …….. …….. …….. ……

The form below for SMRL use only

AP Film of Specimen = Neg + ++ +++

Glycerol Date …/…….	**Pyruvate Date** …/…….	**MGIT/MB Date** …/…….	**BA - NG** …….
Sub to: -	**Date sub'd: -** / /		**BA - Contaminated**
ZN Result: - E B D S / M / L			**Sens required: -**

Date										
Action										
Date										
Action										
Date								……/……/…….		
Action								Growth E or D	Pigment Y / N	For SEQ

Culture Identified as: - **Date: -** ……..……/……/……

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